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Abstract: Arrhythmogenic cardiomyopathy (ACM) is a familial disease, with approximately 60% of patients displaying a pathogenic variant. The majority of genes linked to ACM code for components of the desmosome: plakophilin-2 (PKP2), desmoglein-2 (DSG2) and desmocollin-2 (DSC2), plakoglobin (JUP) and desmoplakin (DSP). Genetic variants involving the desmosomes are known to cause dysfunction of cell-to-cell adhesions and intercellular gap junctions. In turn, this may result in failure to mechanically hold together the cardiomyocytes, fibrofatty myocardial replacement, cardiac conduction delay and ventricular arrhythmias. It is becoming clearer that pathogenic variants in desmosomal genes such as PKP2 are not only responsible for a mechanical dysfunction of the intercalated disc (ID), but are also the cause of various pro-arrhythmic mechanisms. In this review, we discuss in detail the different molecular interactions associated with desmosomal pathogenic variants, and their contribution to various ACM phenotypes.

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Arrhythmogenic cardiomyopathy: An in-depth look at molecular mechanisms and clinical correlates ☆,☆☆

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ABSTRACT

Arrhythmogenic cardiomyopathy (ACM) is a familial disease, with approximately 60% of patients displaying a pathogenic variant. The majority of genes linked to ACM code for components of the desmosome: plakophilin-2 (*PKP2*), desmoglein-2 (*DSG2*) and desmocollin-2 (*DSC2*), plakoglobin (*JUP*) and desmoplakin (*DSP*). Genetic variants involving the desmosomes are known to cause dysfunction of cell-to-cell adhesions and intercellular gap junctions. In turn, this may result in failure to mechanically hold together the cardiomyocytes, fibrofatty myocardial replacement, cardiac conduction delay and ventricular arrhythmias. It is becoming clearer that pathogenic variants in desmosomal genes such as PKP2 are not only responsible for a mechanical dysfunction of the intercalated disc (ID), but are also the cause of various pro-arrhythmic mechanisms. In this review, we discuss in detail the different molecular interactions associated with desmosomal pathogenic variants, and their contribution to various ACM phenotypes.

Introduction

Arrhythmogenic cardiomyopathy (ACM) is a progressive heritable cardiac condition encompassing a broad spectrum of phenotypes. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is the most classical form of ACM. The disease is one of the leading causes of ventricular arrhythmias and may lead to sudden cardiac death (SCD) in young, athletic people. SCD can occur as the first disease manifestation even before structural changes manifest in up to 11% of index cases [1]. The natural history of ACM has four phases: an early *concealed phase*, during which structural abnormalities are undetectable, but can put the patients, especially athletes who practice strenuous exercise, at an increased risk of SCD [2]. The second phase is an *overt electrical disorder* with ECG abnormalities such as inverted T-waves, typically in right precordial leads, and arrhythmias with left bundle branch block (LBBB) morphology, and hence arising from the right ventricle (RV). Structural changes are mostly confined to specific RV regions and are best detected by cardiac magnetic resonance (MRI) or by strain evaluation in echocardiography. The following phase typically shows an *extension of the RV disease* with a progressive loss of healthy myocardium and fibrofatty replacement [3] leading to a RV global dysfunction. Finally, the disease progresses into *end-stage heart failure* with biventricular involvement.

There are also non-classical forms of ACM showing atypical clinical presentation, which have only been reported in recent years. One is a left ventricle (LV)-dominant disease (ALVC) with limited or absent RV involvement, and another is a so-called biventricular form, in which there is an early involvement of both ventricles. Although the natural history of ALVC awaits further elucidation, its clinical profile is clearly distinct from ARVC. In this disease, arrhythmias typically have a right bundle branch block (RBBB) morphology and LV impairment/dilation without RV involvement can be detected in up to 30% of cases [4]. The biventricular form, on the other hand, shows parallel involvement of both ventricles starting in the early stages. Clinically, it may range from a mild disease with localized structural abnormalities to an advanced disease with biventricular dilation and/or systolic

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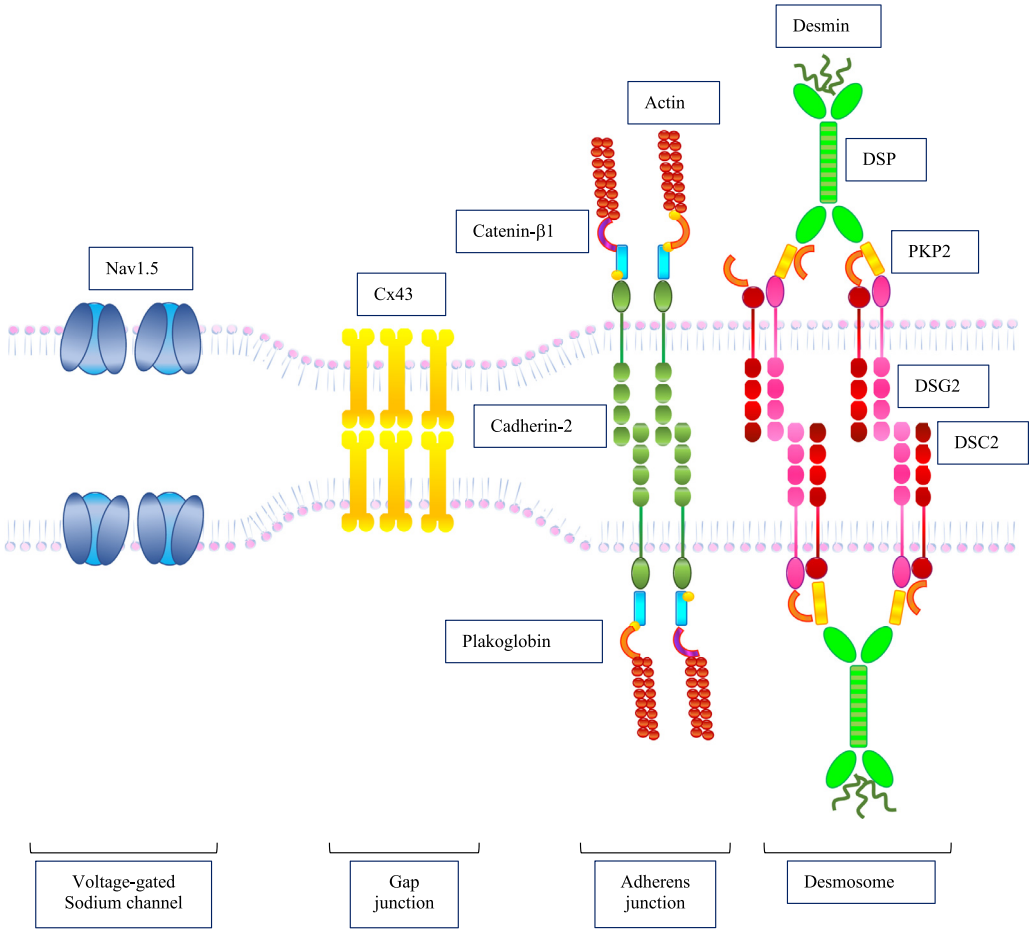

Fig. 1. *The intercalated Disc.*
The ID is composed of three structures: desmosomes, adherens junctions and gap junctions. These regulate respectively the mechanical and electrical continuity of cardiac myocytes, with desmosomes being involved in a crosstalk between adherens and gap junctions [2].

impairment. The electrical phenotype suggests ventricular arrhythmias of biventricular origin [5].

Genetic background and clinical phenotypes

ACM is known to be a familial disease, with approximately 60% of patients carrying a genetic pathogenic variant. It is commonly considered as a disease of the intercalated disc (ID), the structure in charge of the end-to-end contact of cardiac myocytes and implied in mechanical and electrical coupling. (Fig. 1) The majority of genes linked to ACM code for components of the desmosomes: plakophilin-2 (*PKP2*), desmoglein-2 (*DSG2*) and desmocollin-2 (*DSC2*), plakoglobin (*JUP*) and desmoplakin (*DSP*). In addition, there are non-desmosomal genes such as the transmembrane protein 43 (*TMEM43*), desmin (*DES*), phospholamban (*PLB*), N-cadherin (*CDH2*), sodium voltage-gated channel alpha subunit 5 (*SCN5A*), titin (*TTN*) and transforming growth factor 3 beta (*TGF3β*) [6] which have also been shown to cause ACM. While most phenotypes are associated with autosomal dominant transmission, there are two forms that are autosomal recessive. A homozygous mutation in plakoglobin causes Naxos disease, which presents with both cardiac and cutaneous involvement and onset of symptoms starting in childhood. Carvajal Syndrome is another autosomal recessive form, caused by mutations in DSP and also has a cardiocutaneous phenotype, characterized by woolly hair, palmoplantar keratoderma and heart disease [3]. Typical surface ECG abnormalities in ARVC include epsilon waves, QRS prolongation and inverted T waves in the right precordial leads (V1-V3), and arrhythmias of RV origin. Even though structural abnormalities in the RV are the most dominant, LV involvement occurs with disease progression [2]. Pathogenic variants in *PKP2* are usually associated with the classical form of the RV disease [7]. There may be LV involvement in late-stage *PKP2* disease, but this is much less prominent than what can be seen in other genetic forms such as the ones linked to *DSG2* or *DSC2*, which show prominent LV involvement even during early disease. On the other hand, *DSP* pathogenic variants have been associated with ALVC. In this case, clinical features include inferolateral T wave inversions, arrhythmias of LV origin and prominent late gadolinium enhancement (LGE) of the LV myocardium. Of note, cases with multiple heterozygous mutations tend to have a more severe phenotype than cases with a single mutation.

Genetic mutations involving the desmosomes are known to cause dysfunction of cell-to-cell adhesions and intercellular gap junctions. These result in failure to mechanically hold together the cardiomyocytes, fibrofatty myocardial replacement, cardiac conduction delay and ventricular arrhythmias. In this review, we discuss the different molecular interactions associated to desmosomal mutations, which contribute to various ACM phenotypes.

Desmosomal mutations and molecular interactions: mechanisms of arrhythmogenesis

The central role of PKP2

The majority of genotype-positive cases of ARVC are due to *PKP2* pathogenic variants. PKP2 is mainly known for its

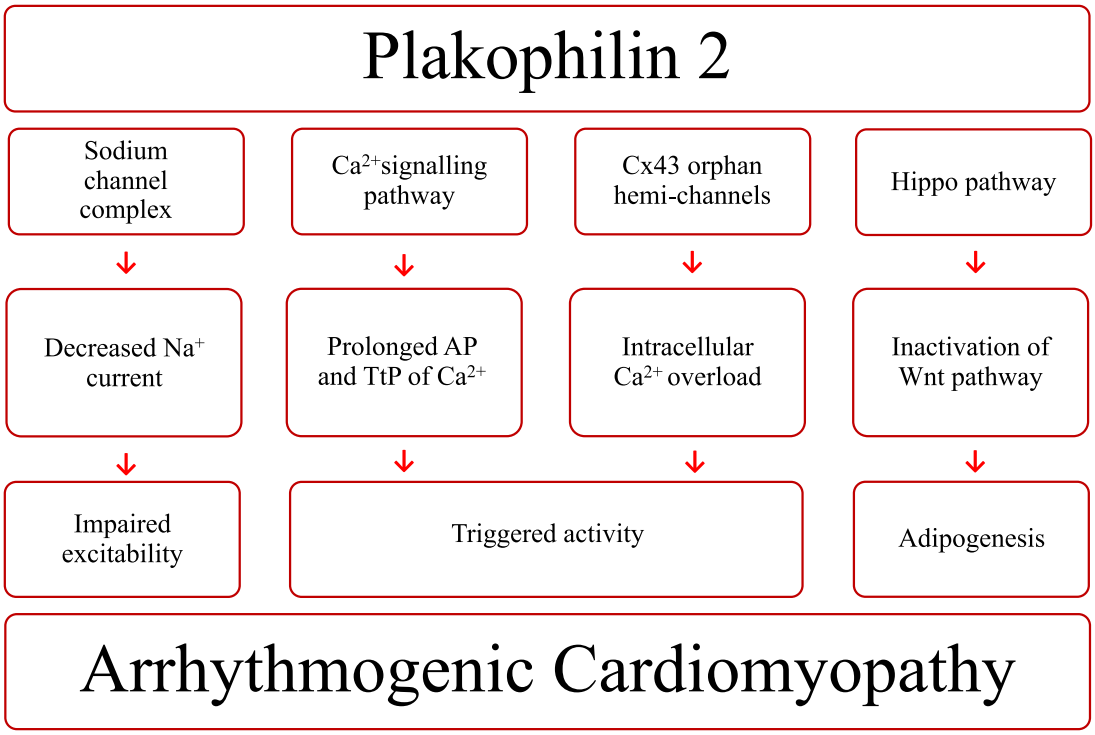

Fig. 2. *The pivotal role of PKP2.*
The malfunction of the *PKP2* protein causes a chain of events, which may lead to arrhythmias through various pathways. • Reduced *PKP2* determines an altered $Na_{v1.5}$ interaction with ancillary proteins such as Ankyrin G, which is required for $Na_{v1.5}$ trafficking [13]. • *PKP2*-cKO cardiomyocytes present an increase in amplitude, prolonged duration and increase in time to peak of calcium transients and a prolonged action potential duration. • Ca^{2+} homeostasis imbalance coincided with a Cx43-dependent increase in RV membrane permeability. Ca^{2+} overload seems to be facilitated by increased availability of Cx43 hemichannels in the sarcolemma, increasing its arrhythmogenic potential.

mechanical functions: It provides the lateral stabilizing force, which facilitates cell-to-cell adhesion together with the desmosomal intermediate filament [2]. Nevertheless, this definition has been revised in recent years in order to include a more pleiotropic role of this protein: on top of its role in cellular adhesion, it is responsible for the regulation of multiple molecular pathways as a part of the connexome complex [8]. The malfunction of the PKP2 protein causes a chain of events that may lead to the disruption of intracellular signaling, of electrophysiology and of the cardiac transcription program [9].

It has been suggested that arrhythmias are a consequence of reentry mechanisms around anatomical obstacles due to patchy cardiomyocyte loss and fibrofatty infiltration. However, while this certainly cannot be excluded, it is now clear that life-threatening ventricular arrhythmias often occur in the early phase of the disease, before any overt structural damage can be found [5]. Studies aimed at discovering the relation between a mutant *PKP2* and arrhythmias proved that *PKP2* is essential to maintain gap junction integrity [10]. While the mechanisms underlying this arrhythmogenicity still remain unclear, there is now evidence that various pathways are involved (Fig. 2).

PKP2 and the sodium channel complex

One of the first molecular interactions described in ACM was the one between *PKP2* and the voltage gated sodium channel (Nav 1.5): they are both located within the cardiac ID, and rat cardiomyocytes silenced for *Pkp2* showed reduced Nav 1.5 levels and sodium current density [11]. Following this path, Cerrone et al. showed in a murine model of *Pkp2* haploinsufficiency that sodium current amplitude was decreased, and the steady-state inactivation had a negative shift. Furthermore, macropatch recordings of cardiomyocytes haploinsufficient for *Pkp2* showed a selectively decreased sodium current in the region of the ID. The reduced abundance of *Pkp2* in this model was shown to correlate with increased separation between the microtubule plus-end tracking protein EB-1 and sites rich with N-cadherin [12]. This led to the hypothesis that *Pkp2* is part of a molecular complex that seizes the microtubules plus-end at the ID and allows passage of its contents. While this evidence alone does not explain why there is a change of gating and kinetics of the sodium current in the model, it is possible that reduced *Pkp2* determines an altered $Na_{v1.5}$ interaction with ancillary proteins such as Ankyrin G, which is required for $Na_{v1.5}$ trafficking [13]. Interestingly, a reduction of Nav 1.5 expression has also been detected in human tissue samples (from the RV free wall postmortem or from RV septal biopsies) from ARVC patients with PKP2 variants, as Noorman et al. showed substantial disturbance of the immunoreactive signals for Nav 1.5, Cx43 and PKG [14]. Similar results were shown in a cohort of patients with heterozygous DSP variants by Gomes et al., where in two tissue samples, Nav 1.5 was localized away from the ID. Furthermore, on electrophysiological mapping there were conduction delays showed by shorter coupling intervals and proven by activation delay curves, especially in the RVOT [15].

However, it is unlikely that sodium channel dysfunction is the only mechanism for the occurrence of spontaneous arrhythmias, but rather it may contribute to arrhythmogenesis in the early stages of ARVC through additional electrophysiological factors.

Pkp2: Ca^{2+} handling and Cx43 interaction

Dysfunctional calcium handling has been proposed as a mechanism for arrhythmogenesis in ACM more recently. Cerrone et al. [16] generated a cardiac restricted, tamoxifen-activated conditional Pkp2 knock-out mouse model (Pkp2-cKO), which manifests a phenotype of progressive ACM starting with RV predominance and

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subsequently showing biventricular involvement. Transcriptome analysis showed that the calcium signaling pathway was affected, generating lower transcripts and lower protein levels of *Ank2* (coding for ankyrin-B), *Ryr2* (coding for the cardiac ryanodine receptor), *Trdn* (coding for triadin) and *Cacna1c* (coding for $Ca_V1.2$). Functional studies showed the Pkp2-cKO cardiomyocytes present a surge in amplitude, prolonged duration and increase in time to peak of calcium transients and a prolonged action potential duration. The loss of Pkp2 also facilitated early and delayed afterdepolarizations, likely sufficient to predispose to ventricular arrhythmias. Interestingly, all these pro-arrhythmic alterations in the Ca^{2+} handling pathways manifested since the early disease stages, before the mouse model presented overt structural cardiomyopathy. Further studies [17] in this model showed that loss of Pkp2 causes an accumulation of Ca^{2+} in three intracellular compartments: the junctional sarcoplasmic reticulum, the cytoplasm and the mitochondria. Moreover, Ca^{2+} homeostasis imbalance was found to be sensitive to reduced expression of Cx43 and coincided with a Cx43-dependent increase in RV membrane permeability. Possibly, loss of desmosomal integrity causes a loss of stability of the neighboring gap junctions with consequent increase of orphan Cx43 hemichannels in the perinexus.

Reduced expression of Cx43 at the ID has also been shown histologically in ACM patients: Kaplan et al. reported that a 7-year-old patient with genotypically confirmed Naxos disease, but no overt structural heart disease (neither at echography, nor histologically), indeed showed significantly reduced myocardial Cx43 expression at the ID [18]. Furthermore, similar results were shown by Gomes et al. who detected marked downregulation of Cx43 at the ID in myocardial biopsies from three patients carrier of DSP heterozygous variants in the absence of overt structural disease [15]. It is important to note that Cx43 occupies both the ID and the mitochondrial membrane and recent studies have shown how mitochondrial Cx43 hemichannels participate in mitochondrial Ca^{2+} entry [19]. Under pathologic conditions, mitochondrial Ca^{2+} overload causes reactive oxygen species (ROS) production, which interferes with the sarcoplasmic reticulum Ca^{2+} cycling through oxidative stress induction and elevates the cytosolic Ca^{2+} levels [20], which in turn activates several arrhythmogenic pathways and causes triggered activity in the heart. In the *Pkp2*-cKO mouse, intracellular Ca^{2+} overload seems to be facilitated by increased availability of Cx43 hemichannels in the sarcolemma [17]. A recent work from our group [21] postulated that a surge in mitochondrial permeability, via mitochondrial Cx43 (MtCx43) channels, would cause mitochondrial Ca^{2+} overload as a consequence of decreased *Pkp2* expression because of an excessive entry of Ca^{2+} into the mitochondria. While further studies are certainly needed, this draws a phenotypical picture in which the loss of *Pkp2* not only creates a RV-predominant cardiomyopathy, but also possibly results in an arrhythmogenic substrate, mostly in the RV ventricle, that precedes the cardiomyopathy (Fig. 3).

The role of DSG2

A *Dsg2* mutant model developed by Pilichou et al. [22] showed an expansion of the ID in proximity of the area composita, which coincided with slowing of conduction, and a consequent reduction in action potential upstroke velocity, stemming from a decrease in the cardiac I_{Na} density. The mutated N271S residue, which is positioned between the second and third extracellular cadherin domains of *Dsg2* is known to be of utmost importance for coordination of Ca^{2+} binding, which on the other hand is essential to the adhesive function of junctional cadherins. Two possible pathogenic mechanisms have been proposed for this model: either the Na^+ channel complex is weakened by the widening of the ID caused by the N271S variant, or the latter causes a conformational change, which challenges the natural interaction between the desmosomal and the Na^+ channel complex regardless of intercellular space widening [23]. Similar to the *Pkp2* mechanism proposed by the Delmar group, using the above mentioned model, Rizzo et al. also provided evidence that *Dsg2* and Nav1.5 interact in vivo, which leads to believe that the presence of a molecular mechanism responsible for conduction slowing and arrhythmia in ARVC prior to overt changes, is indeed possible [24]. This was also confirmed more recently, when El-Battrawy et al. [25] reported how iPSCs-derived cardiomyocytes from an ARVC patient carrying a *Dsg2* variant, presented with multiple ion channel dysfunctions and arrhythmia events, reduced action potential amplitude and V_{max} and a reduced I_{Na}.

The role of DSP

A murine model haploinsufficient for *DSP* showed reduced expression and remodeling of Cx43 at the ID, preceding overt histological abnormalities [15]. The downregulation of Cx43 preceding fibrofatty replacement has further been confirmed in a murine model of cardiac-restricted loss of *Dsp* mice [26]. The *Dsp*-cKO hearts revealed electrical wave front propagation defects that are associated with loss of Cx43. A dose-dependent assessment of *Dsp* loss aimed at confirming that Cx43 loss was a primary consequence of loss of *Dsp*, revealed a dose-dependent knockdown of total and phosphorylated Cx43 levels, independent of any molecular dissociation of the desmosomal as well as the fascia adherens junction complex. This was shown by normal levels of *Pkp2* and N Cadherin in the neonatal cardiomyocytes with *Dsp* knockdown. This loss caused electrical abnormalities prior to the mechanical junction complex dissociation and independently from any histological fibrofatty manifestations [26]. Furthermore, *DSP* is a plakin responsible for tethering intermediate filaments (IF) to desmosomes, and while it does not associate to microtubules (MT) directly, it has been shown to mediate MT reorganization. Patel et al. [27] have shown that *DSP* interaction with the end-binding-1 protein (EB1) of MT (a protein which regulates MT dynamics) is critical to *DSP* regulation of MTs dynamics. The disturbance of this interaction causes mis-localization and malfunction of Cx43: *DSP* or EB1 knockdown or mutations impeding the binding of DSP-EB1 or the membrane localization of this interaction, caused an impaired membrane localization of Cx43 and gap junction function, probably due to either a direct association with EB1 causing disruption or by a mis-localization of *DSP* and thus EB1.

Desmosomal mutations and molecular interactions: fibrofatty infiltration and inflammation

In ACM, part of the myocardium (preferentially the RV) is replaced by fibrotic tissue, which is the histological hallmark of disease. These changes may be accompanied by inflammatory infiltrates, detected in samples from heart autopsies in up to 60–88% of cases [28]. The mechanisms underlying these phenomena are still under investigation and not completely understood.

PKP2 *and fibrosis*

In the presence of cardiac muscle injury, fibroblasts are activated and start differentiating into myofibroblasts, thus initiating the profibrotic process. However, the mechanisms that causes the formation of fibrotic tissue in the myocardium are still not well known. In the setting of ACM, it has been hypothesized that *PKP2* deficiency could facilitate fibrosis through the TGFβ-signaling pathway [29]. Recently non-canonical TGFβ-signaling pathways have been recognized, the most remarkable being the activation

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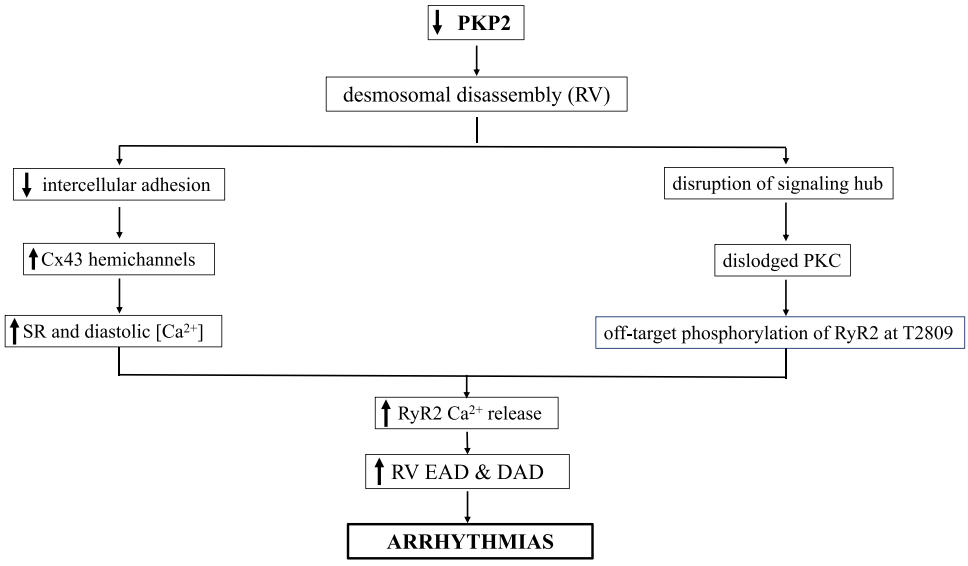

Fig. 3. *PKP2 – RYR2 arrhythmogenesis.*
In the context of the PKP2cko model, sarcoplasmic reticulum (SR) Ca^{2+} release can be modulated both by RyR2 phosphorylation (specifically the phosphorylation on T2809) and increased abundance of connexin43 hemichannels. These pathways facilitate early and delayed after depolarizations, and hence, arrhythmogenesis.

of mitogen-activated protein kinase (MAPK) signaling and generation of ROS [30]. Specifically, this relates to the TGF-β1 P38 MAPK signaling cascade, which elicits the activation of a transcriptional program that is profibrotic. The loss of *PKP2* triggers an increase in TGFβ1 expression, which in turn activates the p38 MAPK cascade, a known supporter of the TGFβ-induced collagen synthesis. The loss of *PKP2* also results in a disruption of the area composita formation with a loss of *DSP* incorporation at cell-to-cell junctions, with an increased solubility and degradation of *DSP* [30]. Even in the presence of a PKP2 deficiency, the reinstatement of *DSP* expression is sufficient to rescue the increased expression of TGF-β1 mRNA and its consequent downstream phosphorylation of p38 MAPK induced by the *PKP2* KD. This points to an interplay between the two proteins. Interestingly, neighboring *PKP2* positive cells show p38-MAPK activation as well, suggesting both the presence of a cell-to-cell interplay between *PKP2* positive and negative cells and of a paracrine pathway recruiting of pro-fibrotic processes.

Adenosine may also play a role as a pro-fibrotic messenger in case of *PKP2* deficiency. It has been shown in previous studies that when adenosine binds to the adenosine 2A receptor (A2AR), this results in an increased collagen deposition in tissues such as skin, lung and liver. In the heart tissue, ATP is swiftly converted into adenosine, which binds to its G-coupled protein receptor (A2AR) [31]. Cerrone et al. explored the role of adenosine in *Pkp2* deficient mouse hearts and showed that in *Pkp2* deficient cells, ATP release was significantly increased, however upon silencing of both *Pkp2* and Cx43, the release of ATP was significantly reduced, which suggests the presence of Cx43 hemichannels as conduit for ATP in *Pkp2* deficient cells [32]. Furthermore, istradefylline (a specific A2AR antagonist) partially interrupted the cardiomyopathic phenotype and reduced collagen deposition, confirming the role of adenosine in the unfolding of ACM.

PKP2 *and adipogenesis*

The Hippo pathway regulates organ size and cell proliferation, survival and differentiation. In the canonical Hippo cascade, it suppresses the YAP pathway, which inhibits the canonical Wnt-pathway through the interaction with cateninβ1. Through the inhibitory Hippo kinase activity, the cateninβ1 translocation to the nucleus is suppressed. In *PKP2* depleted cells this mechanism is supposed to be brought about by the fact that *PKP2* deficiency causes reduction in levels and partial inactivation of PKCα, which is required to inactivate NF2. The activation of NF2 then correlates with the cascade phosphorylation of the Hippo kinases, which in turn prevent the nuclear translocation of βcatenin and thus the activation of the Wnt-pathway [35].

Another candidate for the pro-adipogenic signaling in ACM are PPARs (peroxisome proliferator-activated receptor), in particular PPARγ, a nuclear receptor responsible for the adipogenesis and the differentiation of pre-adipocytes to adipocytes: in a study conducted by Kim et al. [33] hiPSC-CMs mutant for *PKP2* were shown to increase PPARγ expression, decrease cateninβ1 activity and promote lipogenesis.

Desmosomal variants and fibrofatty infiltration

Fibrofatty infiltration was not only shown in the *PKP2* related form. The activation of the Hippo and Wnt-pathways have been proposed as a possible mechanism stimulating fibro-adipogenesis in the setting of desmosomal pathogenic variants [13]. The part played by the Wnt-signaling pathway in fibro-adipogenesis has been described initially for *JUP*. Also known as γ-catenin or PKG (plakoglobin), it shares functional and structural properties with β-catenin, which on the other hand is an activator of the Wnt-signaling pathway via activation of the T cell\lymphoid-enhancing binding transcription factors. Since one of the hallmark features of ACM is fibro-adipogenesis, and suppression of the canonical Wnt/β-catenin pathway is a known cause of this, it is thought that nuclear translocation of *JUP* can inactivate this pathway by competing with β-catenin. This hypothesis was tested initially by Garcia-Gras et al. [34] who showed that the suppression of *DSP* in a cardiac cell line (HL-1 cells) and subsequently in *Dsp* deficient mice led to nuclear localization of *JUP*, its competition with β-catenin, suppression of the Wnt/β-catenin signaling pathway with consequent enhanced myocyte apoptosis, excess fibro-adipogenesis, myocardial dysfunction and ventricular tachycardia. Noorman and colleagues reported decreased ID PKG signal by immunofluorescence in human heart ACM samples, independently from the genetic background [14]. Similar results were shown by Asimaki et al. in a model of neonatal rat cardiomyocytes with the

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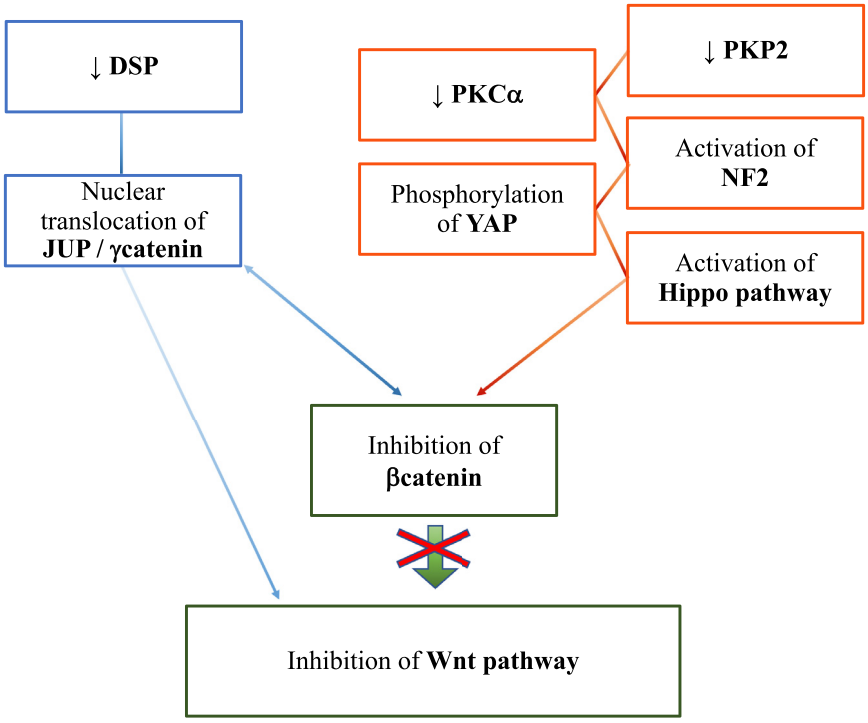

Fig. 4. *Suppression of the Wnt Pathway as a cause for adipogenesis.*
Suppression of the canonical Wnt\β-catenin pathway can lead to enhanced adipogenesis through two possible pathways. • Nuclear translocation of *JUP*, also known as γ-catenin, can inactivate this pathway by competing with β-catenin. • *PKP2* deficiency causes reduction in levels and partial inactivation of PKCα, which then cannot inactivate NF2: this leads to a cascade phosphorylation of the Hippo kinases, which in turn prevent the nuclear translocation of βcatenin and thus the activation of the Wnt-pathway [9].

expression of a truncated version of *Jup* (2057del2). *Jup* was shown to be reduced at the cell-to-cell junctions and abundant in the cell nuclei [35]. Interestingly, the authors also found that the mutant Jup cardiomyocytes showed decreased amounts of SAP97, a regulator protein of Nav 1.5 and Kir2.1, which are the major protein subunits in charge of I_{Na} and I_{k1} currents, respectively. This suggests that altered trafficking of ion channel proteins to the cell membrane may be the cause of the remodeling of the action potential and of the decreased I_{na} and I_{k1} currents. In addition, mutant *Jup* sensitized the cells to the secretion of multiple cytokines such as IL-6 and TNF, suggesting a possible link between desmosomal dysfunction and the known inflammatory response seen in ACM. Furthermore, the SB216763 (SB2) molecule could rescue disease features caused by the expression of *Jup* 2057del2, such as decreased expression of Cx43 or action potential remodeling [35]. This molecule is an inhibitor of GSK3β, and increases canonical Wnt-signaling [36]. Recent studies have shown a dichotomic role of GSK3β: under physiological conditions inhibition of GSK3β empowers Wnt-signaling and disturbs cardiac homeostasis causing myocardial fibrosis, while inhibition of GSK3β in pathologic conditions such as myocardial infarction, results in cardioprotection and downregulation of myocardial fibrosis [37]. On the basis of this, Judge and colleagues have shown how GSK3β localized abnormally at the myocyte junction in two different mouse models of desmosomal dysfunction (*Dsg2*$^{mut/mut}$ mutant mice and *JUP2157del2* mutant mice), at variance with their controls [38]. These models, which were created respectively with an inducible knock-in mutation and a transgenic overexpression of *JUP2157del2* cDNA under the control of the cardiac-specific mouse α-myosin heavy chain (αMHC) promoter, present biventricular fibrosis and focal inflammation, features of the clinical spectrum of ACM. They also show molecular changes (abnormal plakoglobin (PLK) and connexin43 (Cx43) signal at myocyte junctions), when compared to controls. Interestingly, this phenotype was markedly improved by administration of the SB2 molecule mentioned above. Furthermore, the group found the same pattern of abnormal GSK3β distribution at the ID in NRVM expressing mutant forms of *Jup* (JUP2157del2) or *Pkp2* (1851del123) and in paraffin-embedded cardiac tissue sections from ACM patients with known desmosomal gene pathogenic variants. Finally, the pathogenic role of GSK3β was further confirmed by a *Dsg2* mutant mice bred in with a strain of mice with a knock-in mutation in the murine GSK3β gene (constitutively activating GSK3β), which demonstrated an increase in cardiac dysfunction and myocardial fibrosis. Several limitations have to be taken into account when considering these murine models: the *Dsg2* model is not cardiac specific and the possibility of the abnormalities being observed due to disruption of unrelated genes is present. Most of all, since GSK3β is expressed ubiquitously throughout the heart and regulates many different signaling pathways, upstream signaling pathways should be studied further in order to understand their contribution to disease development. Nevertheless, all in all, these data suggest aberrant Wnt/β-catenin signaling and nuclear plakoglobin translocation as possible causal factors for the fibrosis and inflammation seen as part of the ACM phenotype (Fig. 4).

Desmosomal variants and inflammation

Chelko et al. provided further evidence that inflammatory signaling pathways play an essential role in the ACM pathophysiology [39]. In the context of inflammation, it is important to clarify that the immune response present in ACM is made up of two components: on one side the infiltration of the myocardium by cells of the adaptive immune system, such as lymphocytes and macrophages, on the other side the activation of the innate immune system in cardiac myocytes. The role both components may have in the pathogenesis of the disease is still under debate: while it is true that a consistent amount of inflammatory cells can be

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found in the hearts of ACM patients, it is still unclear, whether these are a reactive response to myocardial damage or actually the promotors of it. On the other hand, the activation of the innate component is currently being investigated. In their work the authors showed that the NFκB signaling pathway is activated in three different experimental models of ACM, with different desmosmal variants. This pathway is a master regulator of cellular inflammatory responses, which has clear links to the GSK3β pathway based on evidence indicating that activation of GSK3β promotes inflammation through NFκB [40]. More specifically, they used the same model of NVRM expressing mutant forms of *Jup* (JUP2157del2) described by Judge et al. [38], showing the in vitro activation of the NFκB signaling pathway. Furthermore, they also showed how the NFκB signaling pathway is activated in *Dsg2*$^{mut/mut}$ mice in vivo. When comparing cytokine arrays of these mice with WT mice, substantial expression of multiple cytokines (IL-1β, IFNγ, TNFα) in the hearts of the *Dsg2*$^{mut/mut}$ was shown. The activation of the NFκB signaling pathway was further confirmed in iPSC-cardiomyocytes from a patient carrying a *PKP2* pathogenic variant. This suggests an activation of the innate immune system response in cardiac myocytes, driven at least in part by NFκB signaling.

Conclusions

In ACM, life-threatening ventricular arrhythmias may take place mostly in the pre-clinical phase of the disease, before overt structural disease can be detected. Further research and insights are needed to halt the progression of the disease, especially when considering the mechanisms associated with electrical instability. Overall, the central role played by *PKP2* is confirmed by the fact that it is involved in multiple functions, including complex interactions with other desmosomal proteins. This can in part explain the complexity of the phenotype seen upon its deficiency, which ranges from a purely electrical disease such as catecholaminergic polymorphic VT to an electrical\structural disease such as Brugada Syndrome to a structural disease characterized by mechanical dysfunction associated with arrhythmias as it is the case in ACM.

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